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Cross-transmission of vancomycin-resistant *Enterococcus* in patients undergoing dialysis and kidney transplant

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Abstract

The objective of this study was to investigate the occurrence of vancomycin-resistant *Enterococcus* (VRE) cross-transmission between two patient groups (long-term dialysis and kidney transplant patients). Molecular typing, by automated ribotyping with the RiboPrinter Microbial Characterization System (Qualicon, USA), was used to analyze VRE isolates from 31 fecal samples of 320 dialysis patients and 38 fecal samples of 280 kidney transplant patients. Clonal spread of *E. faecalis* and *E. casseliflavus* was observed intragroup, but not between the two groups of patients. In turn, transmission of *E. gallinarum* and *E. faecium* between the groups was suggested by the finding of vancomycin-resistant isolates belonging to the same ribogroup in both dialysis and transplant patients. The fact that these patients were colonized by VRE from the same ribogroup in the same health care facility provides evidence for cross-transmission and supports the adoption of stringent infection control measures to prevent dissemination of these bacteria.

Key words: Vancomycin-resistant *Enterococcus*; Transmission; Dialysis; Kidney transplant

Introduction

Cross-transmission of multidrug-resistant organisms (MDRO), very often via hands of health care workers, has been a major factor accounting for the increase in MDRO incidence and prevalence, especially for methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci (VRE) in acute care facilities (1). Among the many species of MDRO, enterococci have emerged as increasingly important nosocomial and community-acquired pathogens, since their tolerance against harsh conditions renders them difficult to control in health care environments (2,3).

VRE infection has become a matter of great concern in patients with end-stage renal disease in America and Europe, and it has significantly contributed toward increasing the morbidity and mortality within this population (4). In 2003, VRE was identified in dialysis and renal transplant patients at the Universidade Federal de São Paulo nephrology service. The prevalence of colonization was 14.4% in dialysis patients, with type of dialysis treatment

(hemodialysis vs peritoneal dialysis), hospital admission and length of hospital stay identified as risk factors. In kidney transplant patients, the observed prevalence was 13.6%, and none of these risk factors were identified (4,5). This observation strongly suggests the occurrence of nosocomial cross-transmission.

The aim of this study was to investigate the occurrence of VRE cross-transmission between patients undergoing long-term dialysis and kidney transplant patients at UNIFESP.

Material and Methods

This cross-sectional study was approved by the Research Ethics Committee at UNIFESP. All patients provided written informed consent for storage and later use of their biological materials.

Two to four samples of feces were collected in sterilized receptacles from each patient once a week during a

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1-month period. Thirty-one VRE isolates were obtained from feces of 320 dialysis patients, and 38 VRE isolates from feces of 280 kidney transplant patients (4,5). VRE samples were stored at -20°C in glycerol media at the UNIFESP Special Clinical Microbiology Laboratory until retrieval for the present study.

Between December 2007 and July 2008, these VRE isolates were submitted to further characterization in azide blood agar supplemented with 6 µg/mL vancomycin (6). They were identified at the species level by conventional biochemical tests as described by Facklam et al. (7). Vancomycin resistance was confirmed by disk diffusion and the E-test (AB Biodisk, Sweden) according to Clinical and Laboratory Standards Institute (CLSI) breakpoints (8).

Molecular typing

Molecular typing was performed by automated ribotyping, employing the RiboPrinter Microbial Characterization System (Qualicon, USA). This automated process includes cell lysis, DNA cleavage by restriction enzymes (*EcoRI*), and band separation using electrophoresis gel and the modified Southern blot technique. DNA fragments were hybridized with a labeled universal probe derived from ribosomal RNA from *Escherichia coli*, and bands were detected by means of a luminescent chemical substrate. Images were captured on a camera and electronically transferred to a computer coupled to the ribotyping system. Every line representing sample data was normalized in accordance with a standard marker based on the intensity of the bands. The coefficient of similarity was calculated by the computing system on the basis of the position and relative weight of the bands. All samples with band patterns presenting a coefficient of similarity ≥ 0.90 were included in the same ribogroup. The samples with coefficients of similarity below 0.90 were classified into distinct ribogroups (9).

Dendrogram analysis

The identified ribogroups were exported as a TIFF file and imported into the BioNumerics™ software (Applied Maths, Belgium). The patterns were normalized based on the mobility of standards, and a similarity matrix was created. The clustering was developed on the basis of the unweighted pair group method with arithmetic averages (UPGMA). The Dice correlation coefficient was used to analyze the similarities of the banding patterns. Based on the use of internal controls in the database, pattern optimization and band position tolerance of 0.8 and 1.0, respectively, were allowed.

Results

The following species were identified by automated ribotyping in 31 VRE isolates from dialysis patients: *E. faecalis* (N = 2), *E. casseliflavus* (N = 5), *E. faecium* (N = 9), and *E. gallinarum* (N = 15). In the 38 samples from kidney

transplant patients, *E. faecalis* (N = 10), *E. casseliflavus* (N = 10), *E. faecium* (N = 10), and *E. gallinarum* (N = 8) were observed.

Following automated ribotyping, computational analysis using the BioNumerics™ software was used to compare typing data for all bacterial isolates in order to group the organisms according to degree of similarity. This allows determination of clusters sharing a common source of infection. A similarity coefficient of 80% was selected to define ribogroup clusters after reviewing the epidemiologic data associated with each of the clusters of MDRO clones (10-12). Thirty-five ribogroups were found: *E. faecalis* with 3 ribogroups, *E. casseliflavus* with 14 ribogroups, *E. faecium* with 13 ribogroups, and *E. gallinarum* with 7 ribogroups.

E. faecium 112-S-4 was observed in 4 hemodialysis patients and in 1 transplant patient. As shown in Figure 1A, these samples had a coefficient of similarity $>83\%$, suggesting clonal dissemination. The predominant ribogroup was *E. gallinarum* 112-S-4, which colonized 13 hemodialysis patients and four transplant patients, with a 92% coefficient of similarity, again suggesting clonal dissemination between the two patient groups. Furthermore, ribogroup 116-S-3 observed in one kidney transplant patient showed more than 84% similarity with the 112-S-4 ribogroup, indicating a possible relationship between these samples (data not shown).

The largest ribogroup diversity was observed for *E. casseliflavus* samples. Nevertheless, only one *E. casseliflavus* ribogroup, namely 112-S-4, was detected in two kidney transplant samples (intragroup transmission). It should be noted, however, that dendrogram analysis of the *E. casseliflavus* 112-S-4 ribogroup revealed a coefficient of similarity of 87% with the 115-S-4 ribogroup from hemodialysis patient samples (data not shown). Despite the fact that these are different ribogroups, this finding also supports the notion of cross-transmission between the two patient groups.

Of the *E. faecalis* samples from kidney transplant patients, nine were classified in the same ribogroup, 112-S-4. Only one sample was classified as belonging to a distinct ribogroup, 118-S-6. Interestingly, these ribogroups had a similarity $>87\%$, suggesting cross-transmission of this pathogen within the transplant group. Based on dendrogram analysis, it is possible to confirm intragroup clonal spread of *E. faecalis* in hemodialysis patients, but not between the dialysis and transplant groups (Figure 1B).

Discussion

VRE infection is a growing problem in specific groups of patients. According to the National Healthcare Safety Network, 26% of blood cultures in outpatient dialysis are caused by VRE (13).

The resistance to glycopeptides might be mediated by various gene clusters: *vanA*, *vanB*, *vanC*, *vanD*, *vanE*, *vanG*, and *vanL* (14). *VanA*-related clusters have high-

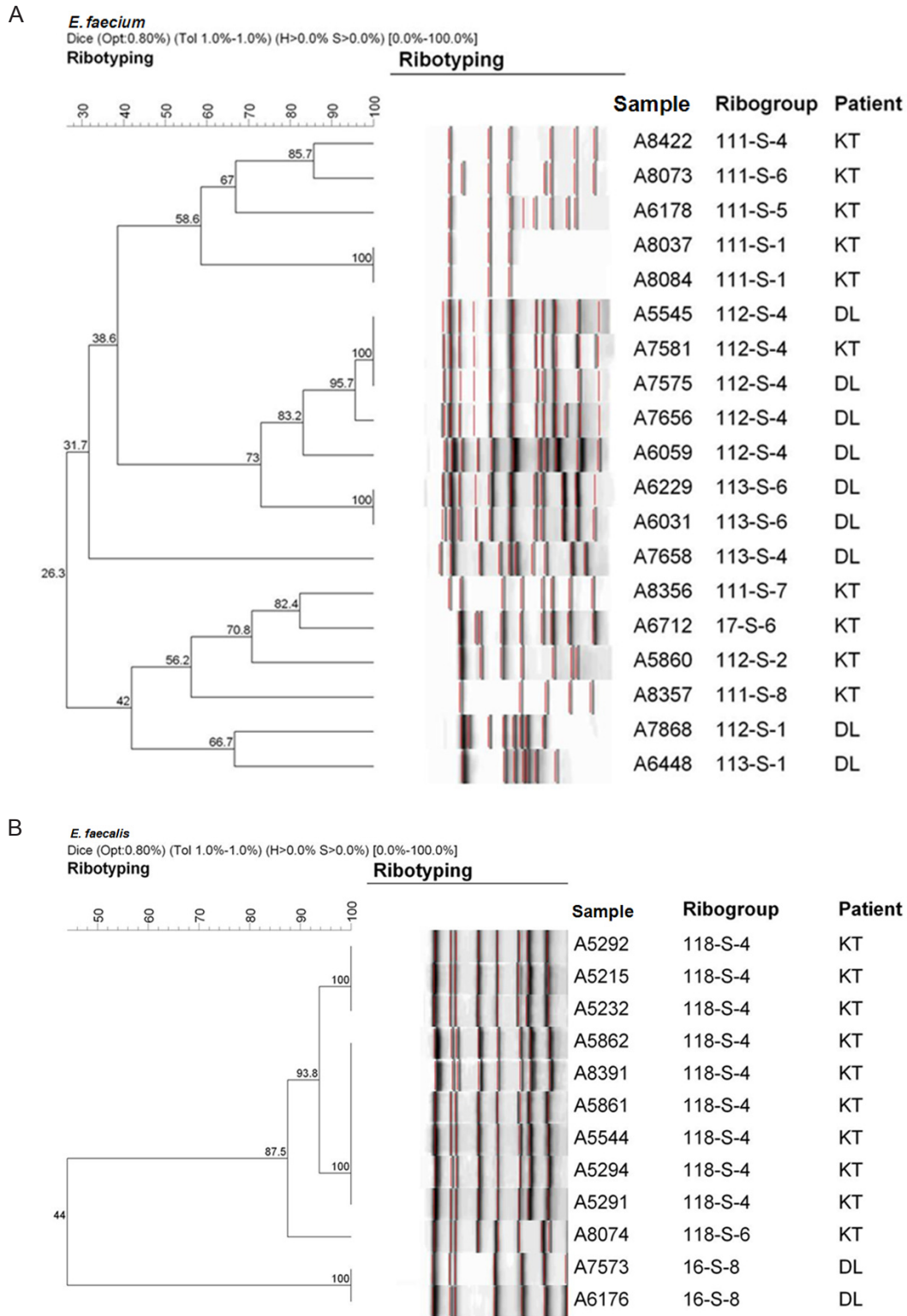


Figure 1. A, Dendrogram analysis of *Enterococcus faecium* ribogroups isolated from dialysis (DL) and kidney transplant patients (KT). B, Dendrogram analysis of *E. faecalis* ribogroups isolated from DL and KT patients.

level resistance to vancomycin and teicoplanin (15). *VanB* isolates were believed to be inducibly resistant to lower levels of vancomycin; however, the range of resistance has been reported to be fairly broad (4 to ≥ 1000 $\mu\text{g/mL}$) and susceptibility to teicoplanin is retained (16).

Gene resistance is mediated by mobile elements (plasmids) that can be transferred from one strain of *Enterococcus* to another. *VanA*- and *vanB*-resistant phenotypes have been described primarily in *E. faecalis* and *E. faecium*, which are responsible for most enterococcal infections and nosocomial VRE outbreaks, usually carried by gene *vanA*. *E. gallinarum* and *E. casseliflavus* are also occasionally responsible for infections (15).

In the present study, we showed that dialysis and kidney transplant patients were colonized with similar vancomycin-resistant *E. faecium* and *E. gallinarum* ribogroups. VRE has become an important nosocomial pathogen because of its rapid spread, high mortality rates associated with infections, limited options for treatment and the possibility of transferring the *vanA* resistance gene to other more virulent and more prevalent pathogens, such as *S. aureus* (17).

McNeil et al. (18) showed that patients who acquired VRE after transplant had worse outcomes than those with VRE colonization documented before transplant (longer duration of hospitalization and intensive care unit stay and higher mortality rates). Mathematical modeling studies have been used to estimate the impact of active surveillance cultures to control MDRO. One such study evaluating interventions to decrease VRE transmission indicated that use of active surveillance cultures, versus no cultures, could potentially decrease transmission by 39%, and that with pre-emptive isolation plus active surveillance cultures transmission could be decreased by 65% (19).

Our results show the VRE cross-transmission occurred between the two patient groups analyzed, kidney transplant and hemodialysis patients. Patient-to-patient transmission in health care settings has been a major factor accounting for the increase in VRE incidence and prevalence. In a study conducted by Padiglione et al. (20), the analysis of VRE strains isolated in an acute-care hospital suggested that most isolates were non-clonal on pulsed-field gel

electrophoresis (PFGE), and that the strict infection control procedures that were in place at each study site were reasonably effective in preventing the nosocomial transmission of dominant clones. Nevertheless, the fact that some strains had similar PFGE patterns suggests that hospital-related transmission of VRE cannot be ruled out.

In our patients, the finding of *E. faecium* and *E. faecalis* strains with the same or similar molecular profiles by ribotyping strongly suggests patient to patient transmission by a common source, justifying the implementation of strict barrier measures. Freitas et al. (5) observed an unexpectedly high rate of VRE colonization in kidney transplant patients, which is very similar to that observed in intensive care units. Furthermore, a high rate of VRE colonization was observed in outpatients, raising the question of whether surveillance should be extended to recently admitted patients. Due to the high costs involved in surveillance culture, this strategy could be reserved for patients with known risk factors, or for those with a higher probability of transmission.

Some measures must be established to prevent the emergence and transmission of VRE. These include environmental measures such as cleaning and disinfection of patient care areas and equipment, single-patient use of non-critical equipment, and decolonization therapy when appropriate. Other useful measures include adequate dimensioning of the nursing staff, communication systems, education and training of medical and other healthcare personnel, judicious antibiotic use, performance improvement processes to ensure adherence to recommended infection control procedures, and comprehensive surveillance for application of MDRO infection control precautions during patient care (19).

Cross-transmission of VRE was observed between two groups of patients at a university hospital. It is possible that the dissemination of MDRO reported in this study may have been caused by factors that can be modified so as to benefit patients, staff, and the institution.

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